

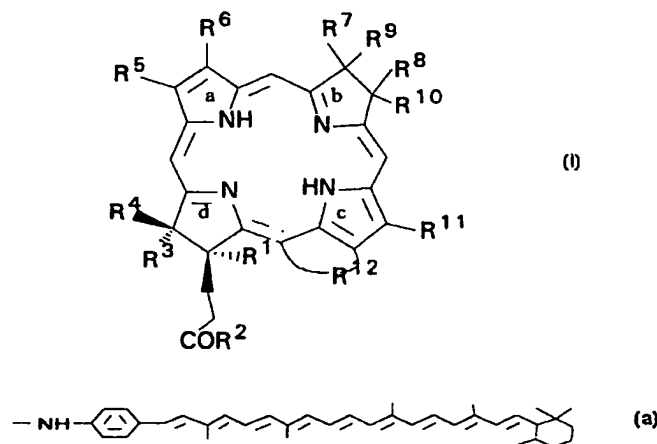


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(54) Title: CAROTENE ANALOG OF PORPHYRINS, CHLORINS AND BACTERIOCHLORINS AS THERAPEUTIC AND DIAGNOSTIC AGENTS



(57) Abstract

A carotene conjugate of formula (I), where R¹ is hydrogen or methyl; R² is formula (a); R³, R⁴ and R⁵ are independently, hydrogen, methyl or ethyl; R⁶ and R⁷ are independently -R¹³, -OR¹³, -C(R¹⁶)(O), -C(R¹⁶)₂OR¹³, -CH=CHR¹³, or -(CH₂)R¹⁴; R⁸ is -R¹³, -OR¹³, -C(R¹⁶)(O), -C(R¹⁶)₂OR¹³, -CH=CHR¹³, or -(CH₂)R¹⁴ or taken with R¹⁰ is -O; R⁹ is -R¹³, -OR¹³, -C(R¹⁶)(O), -C(R¹⁶)₂OR¹³, -CH=CHR¹³, or -(CH₂)R¹⁴ or taken with R¹⁰ is a chemical bond; R¹⁰ is -R¹³, -OR¹³, -C(R¹⁶)(O), -C(R¹⁶)₂OR¹³, -CH=CHR¹³, or -(CH₂)R¹⁴ or taken together with R⁹ is a chemical bond or taken with R⁸ is -O; R¹¹ is R¹³, or -OR¹³; R¹² is -C(R¹³)₂C(Y)-, -C(O)O(O)C-, -C(NR¹³)O(O)C-, or -C(O)N(R¹⁵)-C(O)-; R¹³ is, independently at each occurrence, hydrogen or lower alkyl of from 1 through about 10 carbon atoms; R¹⁴ is an amino acid residue, R¹⁵ is -R¹³, -R¹⁴, or -C(O)NHR¹³; R¹⁶ is, independently at each occurrence, hydrogen or lower alkyl of 1 to about 4 carbon atoms and Y is -O, -S, or 2H-.

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CAROTENE ANALOG OF PORPHYRINS, CHLORINS AND BACTERIOCHLORINS AS THERAPEUTIC AND DIAGNOSTIC AGENTS

Background of the Invention

In recent years, the use of fluorescence spectroscopy has been explored for diagnosis of cancer. Infrared imaging (IRI) using a spectroscopic agent, has several advantages over other in vivo techniques in that the technique is non-invasive and under proper conditions can give deep penetration and quantitative results. The complete profile of uptake, retention and elimination of needed spectroscopic agents can be followed within a single laboratory animal thus reducing the number of animals required in preclinical trials.

The requirements for an ideal spectroscopic agent needed for infrared imaging techniques are as follows: i) it should preferentially localize in tumor cells; ii) it should have high fluorescent efficiency; iii) it should not produce phototoxicity or other adverse effects in a patient; iv) it should be easy to synthesize; v) it should be chemically pure; and vi) it should have a long wave length emission so that deep seated tumors can be detected.

Porphyrins, chlorins, and bacteriochlorins including their analogs and derivatives have recently found superior utility as photodynamic compounds for use in diagnosis and treatment of disease, especially certain cancers. These compounds have also found utility in treatment of psoriasis and papillomatosis.

Such derivatives include dimers and trimers of these compounds. Permissible derivatives also include ring variations of these compounds; provided that, the central

sixteen sided four nitrogen heterocycle of these compounds remains intact. Chlorophyllins, purpurins and pheophorbides and their derivatives are, therefore, included within "porphyrins, chlorins, and bacteriochlorins and their derivatives and analogs". Such derivatives include modifications of substituents upon these ring structures.

Numerous articles have been written on this subject, e.g. "Use of the Chlorophyll Derivative Purpurin-18, for Synthesis of Sensitizers for Use in Photodynamic Therapy", Lee et al., J.Chem.Soc., 1993, (19) 2369-77; "Synthesis of New Bacteriochlorins And Their Antitumor Activity", Pandey et al., Biology and Med. Chem. Letters, 1992; "Photosensitizing Properties of Bacteriochlorophyllin a and Bacteriochlorin a, Two Derivatives of Bacteriochlorophyll a", Beems et al., Photochemistry and Photobiology, 1987, v. 46, 639-643; "Photoradiation Therapy. II. Cure of Animal Tumors With Hematoporphyrin and Light", Dougherty et al., Journal of the National Cancer Institute, July 1975, v. 55, 115-119; "Photodynamic therapy of C3H mouse mammary carcinoma with haematoporphyrin di-esters as sensitizers", Evensen et al., Br. J. Cancer, 1987, 55, 483-486; "Substituent Effects in Tetrapyrrole Subunit Reactivity and Pinacol-Pinacolone Rearrangements: *VIC*-Dihydroxychlorins and *VIC*-Dihydroxybacteriochlorins" Pandey et al., Tetrahedron Letters, 1992, v. 33, 7815-7818; "Photodynamic Sensitizers from Chlorophyll: Purpurin-18 and Chlorin *p₆*", Hooper et al., 1988, v.48, 579-582; "Structure/Activity Relationships Among Photosensitizers Related to Pheophorbides and Bacteriopheophorbides", Pandey et al., Bioorganic and Medicinal Chemistry Letters, 1992, v 2, 491-496; "Photodynamic Therapy Mechanisms", Pandey et al., Proceedings Society of Photo-Optical

Instrumentation Engineers (SPIE), 1989, v 1065, 164-174; and "Fast Atom Bombardment Mass Spectral Analyses of Photofrin II® and its Synthetic Analogs", Pandey et al., Biomedical and Environmental Mass Spectrometry, 1990, v. 19, 405-414. These articles are incorporated by reference herein as background art.

Numerous patents in this area have been applied for and granted world wide on these photodynamic compounds. Reference may be had, for example to the following U.S. Patents which are incorporated herein by reference: 4,649,151; 4,866,168; 4,889,129; 4,932,934; 4,968,715; 5,002,962; 5,015,463; 5,028,621; 5,145,863; 5,198,460; 5,225,433; 5,314,905; 5,459,159; 5,498,710; and 5,591,847.

At least one of these compounds "Photofrin®" has received approval for use in the United States, Canada and Japan. Others of these compounds are in clinical trials or are being considered for such trials.

The term "porphyrins, chlorins and bacteriochlorins" as used herein is intended to include their derivatives and analogs, as described above, and as described and illustrated by the foregoing articles and patents incorporated herein by reference as background art.

Such compounds have been found to have the remarkable characteristic of preferentially accumulating in tumors rather than most normal cells and organs, excepting the liver and spleen. As a result, many tumors can be detected at an early stage due to the light fluorescing nature of the compounds in the tumors. Furthermore, many such tumors can be killed because the compounds may be activated by light to become tumor toxic.

Unfortunately such compounds, as might be expected, are not without some side affects. One of the most annoying, though usually manageable, side effects is the fact that patients exposed to these compounds become sensitive to light. As a result, after treatment, a patient must restrict exposure to light, especially sun light, by remaining indoors as much as possible, by use of dark glasses and by careful use of sun screens.

It would therefore be desirable to have a photodynamic compound, as described above, which continues to be effective as a diagnostic aid, yet having reduced photosensitizing qualities.

Brief Description of the Drawings

Figure 1 shows a schematic reaction scheme for preparation of HPPH-carotene conjugate from HPPH and aminocarotene.

Figure 2 shows a schematic reaction scheme for preparation of purpurin imide-carotene conjugate from purpurine imide and aminocarotene.

Figure 3 is a graph showing uptake of HPPH at 24 hours post injection.

Figure 4 is a graph showing uptake of purpurin imide at 24 hours post injection.

Figure 5 is a graph showing uptake of HPPH-carotene conjugate at 3 hours post injection.

Figure 6 is a graph showing uptake of HPPH-carotene conjugate at 24 hours post injection.

Brief Description of the Invention

In accordance with the invention, photodynamic compounds are provided which continue to have desired photodiagnostic qualities but with reduced photosensitizing characteristics for the patient.

In particular, such compounds have been surprisingly found which are relatively simple derivatives of many known effective photodynamic compounds.

Such compounds are carotene conjugates of photosensitizers selected from the group consisting of porphyrins, chlorins and bacteriochlorins. It was especially surprising that the carotene conjugation does not significantly reduce the tumor uptake characteristics of the photosensitizer.

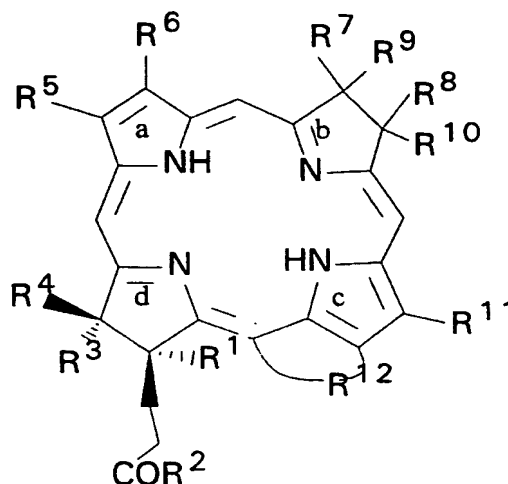
"Carotene" as used herein means carotene and any slight modification thereof which does not adversely affect the phototoxicity reducing quality, e.g. the singlet oxygen quenching characteristic, of the carotene.

The compounds of the invention can be readily made from essentially any of the porphyrins, chlorins and bacteriochlorins discussed above in background art; provided that, such compound has a free carboxylic acid group or a free carboxylic acid ester group or a free carboxylic acid salt group, (collectively "carboxy functionality"). Most of the porphyrins, chlorins, and bacteriochlorins discussed in the background of the invention have such a group.

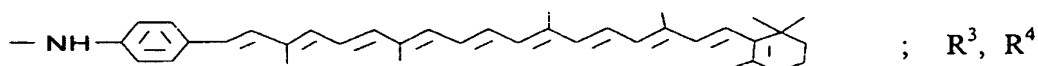
In preparing the compounds of the invention, the carboxy functionality is activated by reaction with a suitable carotene reactive substance and then reacted with the carotene. For example, the carboxy functionality may be reacted with a

carbodiimide and then condensed with amino carotene to obtain the desired carotene conjugate.

A generic formula for many such carotene conjugates is:



where R^1 is hydrogen or methyl; R^2 is:



and R^5 are independently, hydrogen, methyl or ethyl; R^6 and R^7 are independently - R^{13} , - OR^{13} , - $C(R^{16})(O)$, - $C(R^{16})_2OR^{13}$, - $CH=CHR^{13}$, or $-(CH_2)R^{14}$; R^8 is - R^{13} , - OR^{13} , - $C(R^{16})(O)$, - $C(R^{16})_2OR^{13}$, - $CH=CHR^{13}$, or $-(CH_2)R^{14}$ or taken with R^{10} is $=O$; R^9 is - R^{13} , - OR^{13} , - $C(R^{16})(O)$, - $C(R^{16})_2OR^{13}$, - $CH=CHR^{13}$, or $-(CH_2)R^{14}$ or taken with R^{10} is a chemical bond; R^{10} is - R^{13} , - OR^{13} , - $C(R^{16})(O)$, - $C(R^{16})_2OR^{13}$, - $CH=CHR^{13}$, or $-(CH_2)R^{14}$ or taken together with R^9 is a chemical bond or taken with R^8 is $=O$; R^{11} is R^{13} , or $-OR^{13}$; R^{12} is $-C(R^{13})_2C(Y)-$, $-C(O)O(O)C-$, $-C(NR^{13})O(O)C-$, or $-C(O)N(R^{15})-C(O)-$; R^{13} is, independently at each occurrence, hydrogen or lower alkyl of from 1 through about 10 carbon atoms; R^{14} is an amino acid residue, R^{15} is $-R^{13}$, -

R^{14} , or $-C(O)NHR^{13}$; R^{16} is, independently at each occurrence, hydrogen or lower alkyl of 1 to about 4 carbon atoms and Y is $=O$, $=S$, or $2H$.

Detailed Description of the Invention

Specific embodiments illustrating the compounds of the invention are as follows:

1. R^1 is H, R^3 is $-CH_3$, R^4 is H, R^5 is CH_3 , R^6 is $-CH(CH_3)O(CH_2)_6CH_3$, R^7 is $-CH_3$, R^8 is $-CH_2CH_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-CH_3$ and R^{12} is $-C(O)CH_2-$.

2. R^1 is H, R^3 is $-CH_3$, R^4 is H, R^5 is CH_3 , R^6 is $-CH(CH_3)O(CH_2)_6CH_3$, R^7 is $-CH_3$, R^8 is $-CH_2CH_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-CH_3$ and R^{12} is $-C(O)N((CH_2)_5CH_3)CH_3-$.

3. R^1 is H, R^3 is $-CH_3$, R^4 is H, R^5 is $-CH_3$, R^6 is $-CH(CH_3)O(CH_2)_6CH_3$, R^7 is $-CH_3$, R^8 is $-CH_2CH_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-CH_3$ and R^{12} is $-C(O)N((CH_2)_3CH_3)CH_3-$.

4. R^1 is H, R^3 is $-CH_3$, R^4 is H, R^5 is $-CH_3$, R^6 is $-CH(CH_3)OCH_3$, R^7 is $-CH_3$, R^8 is $-CH_2CH_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-CH_3$ and R^{12} is $-C(O)N((CH_2)_2CH_3)C(O)-$.

5. R^1 is H, R^3 is $-CH_3$, R^4 is H, R^5 is $-CH_3$, R^6 is $-CH(CH_3)O(CH_2)_2CH_3$, R^7 is $-CH_3$, R^8 is $-CH_2CH_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-CH_3$ and R^{12} is $-C(O)N((CH_2)_2CH_3)C(O)-$.

6. R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is CH_3 , R^6 is $-\text{CH}(\text{CH}_3)\text{OCH}_3$, R^7 is $-\text{CH}_3$, R^8 is $-\text{CH}_2\text{CH}_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}((\text{CH}_2)_5\text{CH}_3)\text{C}(\text{O})-$.

7. R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is CH_3 , R^6 is $-\text{CH}(\text{CH}_3)\text{O}(\text{CH}_2)_2\text{CH}_3$, R^7 is $-\text{CH}_3$, R^8 is $-\text{CH}_2\text{CH}_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}((\text{CH}_2)_5\text{CH}_3)\text{C}(\text{O})-$.

8. R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is CH_3 , R^6 is $-\text{CH}(\text{CH}_3)\text{O}(\text{CH}_2)_5\text{CH}_3$, R^7 is $-\text{CH}_3$, R^8 is $-\text{CH}_2\text{CH}_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}((\text{CH}_2)_2\text{CH}_3)\text{C}(\text{O})-$.

9. R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is CH_3 , R^6 is $-\text{CH}(\text{CH}_3)\text{O}(\text{CH}_2)_5\text{CH}_3$, R^7 is $-\text{CH}_3$, R^8 is $-\text{CH}_2\text{CH}_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}((\text{CH}_2)_5\text{CH}_3)\text{C}(\text{O})-$.

10. R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is CH_3 , R^6 is $-\text{CH}_2\text{CH}_2\text{OR}^{17}$, R^7 is $-\text{CH}_3$, R^8 is $-\text{CH}_2\text{CH}_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}((\text{CH}_2)_5\text{CH}_3)\text{C}(\text{O})-$ and R^{17} is primary or secondary alkyl containing 1 to about 20 carbon atoms.

11. R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is $-\text{CH}_3$, R^6 is $-\text{CH}(\text{CH}_3)\text{OR}^{17}$, R^7 is $-\text{CH}_3$, R^8 is $-\text{CH}_2\text{CH}_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}((\text{CH}_2)_5\text{CH}_3)\text{C}(\text{O})-$ and R^{17} is primary or secondary alkyl containing 1 to about 20 carbon atoms.

12. R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is CH_3 , R^6 is lower alkyl of 1 to 4 carbon atoms or a formal or carbonyl containing group of 1 to 4 carbon atoms, R^7 is $-\text{CH}_3$, R^8 is H, $-\text{OR}^{13}$ or with R^{10} is $=\text{O}$, R^9 is H, $-\text{OR}^{13}$ or with R^{10} forms a chemical bond, R^{10} is ethyl or with R^9 forms a chemical bond or with R^8 is $=\text{O}$, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}(\text{R}^{13})\text{C}(\text{O})-$ or $-\text{C}(\text{O})\text{O}(\text{O})\text{C}-$.

13. R^1 is hydrogen, R^3 is methyl, R^4 is hydrogen, R^5 is methyl, R^6 is $-\text{CH}(\text{CH}_3)\text{OR}^{13}$, R^7 is methyl, R^8 is ethyl, R^9 and R^{10} together form a chemical bond; R^{11} is methyl; and R^{12} is $-\text{CH}_2\text{C}(\text{O})-$.

14. R^1 is hydrogen, R^3 is methyl, R^4 is hydrogen, R^5 is methyl, R^6 is $-\text{CH}=\text{CH}_2$, R^7 is methyl, R^8 is ethyl, R^9 and R^{10} together form a chemical bond; R^{11} is methyl; and R^{12} is $-\text{CH}_2\text{C}(\text{O})-$.

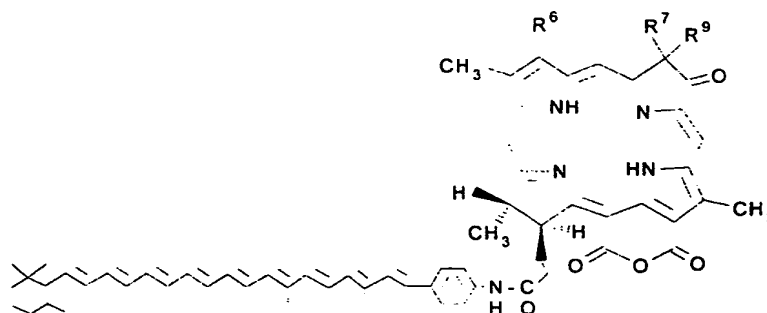
15. R^1 is hydrogen, R^3 is methyl, R^4 is hydrogen, R^5 is methyl, R^6 is $-\text{CH}(\text{CH}_3)\text{O}(\text{CH}_2)_5\text{CH}_3$, R^7 is methyl, R^8 is ethyl, R^9 and R^{10} together form a chemical bond; R^{11} is methyl; and R^{12} is $-\text{CH}_2\text{C}(\text{O})-$.

16. R^1 is hydrogen, R^3 is methyl, R^4 is hydrogen, R^5 is methyl, R^6 is $-\text{CH}=\text{CH}_2$, R^7 is methyl, R^8 is ethyl, R^9 and R^{10} together form a chemical bond; R^{11} is methyl; and R^{12} is $-\text{C}(\text{O})\text{OC}(\text{NR}^{13})-$.

17. R^1 is hydrogen, R^3 is methyl, R^4 is hydrogen, R^5 is methyl, R^6 is $-\text{CH}=\text{CH}_2$, R^7 is methyl, R^8 is ethyl, R^9 and R^{10} are hydrogen; R^{11} is methyl; and R^{12} is $-\text{C}(\text{O})\text{OC}(\text{NR}^{13})-$.

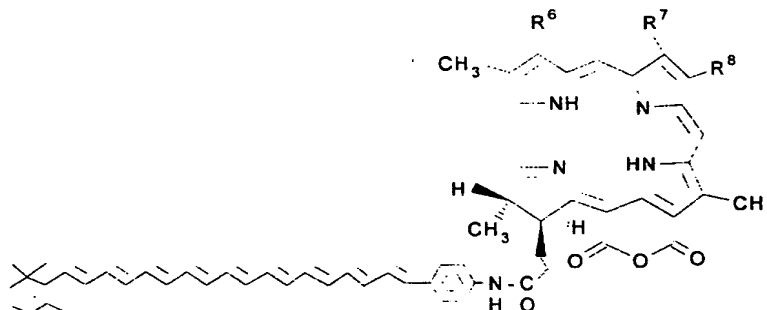
Preferred compounds of the invention may be represented by the following formulas:

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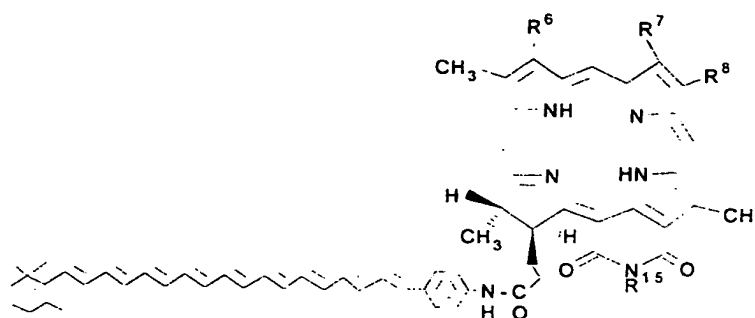
Where R^6 , R^7 , and R^9 are independently hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether or an amino acid residue; provided that, R^6 , R^7 , and R^9 together contain no more than a total of 16 carbon atoms.

2.



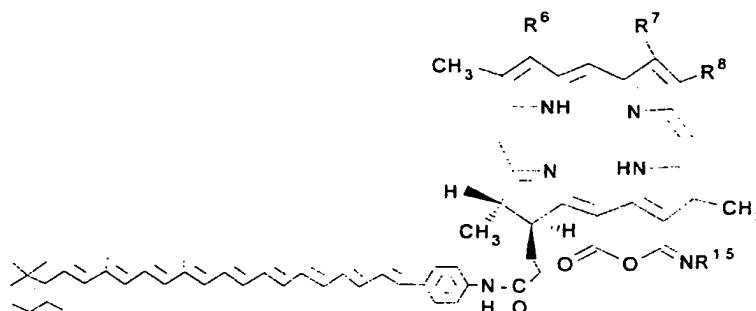
Where R^6 , R^7 , and R^8 are independently hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether or an amino acid residue; provided that, R^6 , R^7 , and R^8 together contain no more than a total of 16 carbon atoms.

3.



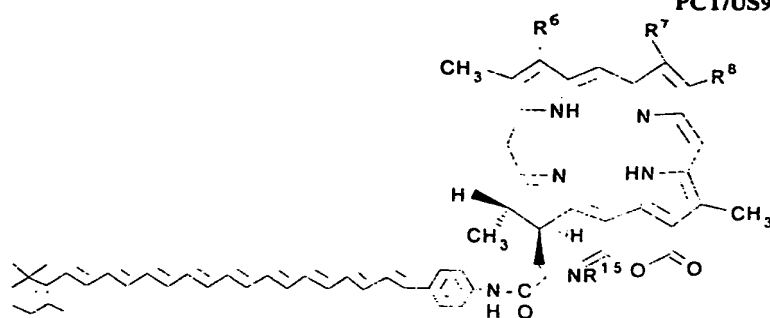
Where R⁶, R⁷, and R⁸ are independently hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether or an amino acid residue; provided that, R⁶, R⁷, and R⁸ together contain no more than a total of 16 carbon atoms and R¹⁵ is hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether, an amino acid residue or – C(O)NHR¹⁸ where R¹⁸ is lower alkyl of from 1 to about 12 carbon atoms.

4.



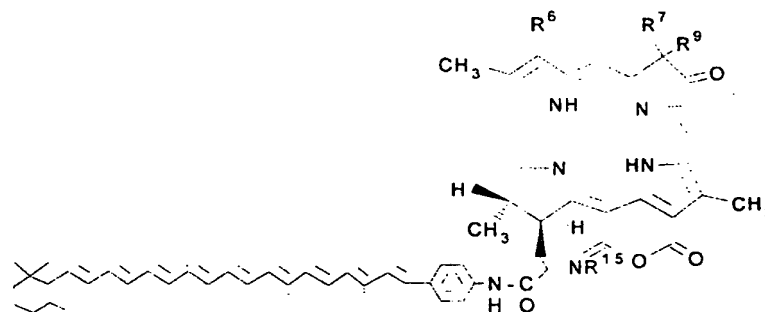
Where R⁶, R⁷, and R⁸ are independently hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether or an amino acid residue; provided that, R⁶, R⁷, and R⁸ together contain no more than a total of 16 carbon atoms and R¹⁵ is hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether, an amino acid residue or – C(O)NHR¹⁸ where R¹⁸ is lower alkyl of from 1 to about 12 carbon atoms.

5.



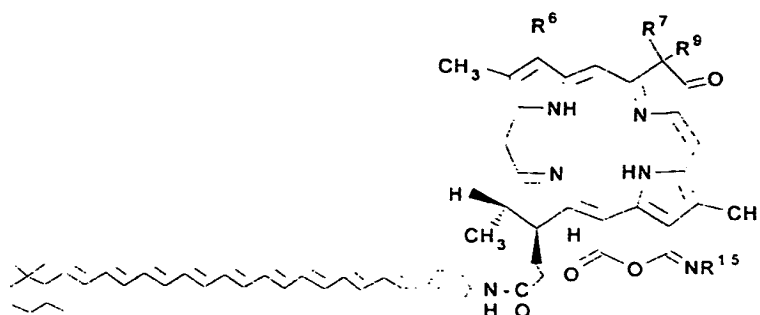
Where R^6 , R^7 , and R^9 are independently hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether or an amino acid residue; provided that, R^6 , R^7 , and R^8 together contain no more than a total of 16 carbon atoms and R^{15} is hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether, an amino acid residue or $-C(O)NHR^{18}$ where R^{18} is lower alkyl of from 1 to about 12 carbon atoms.

6.



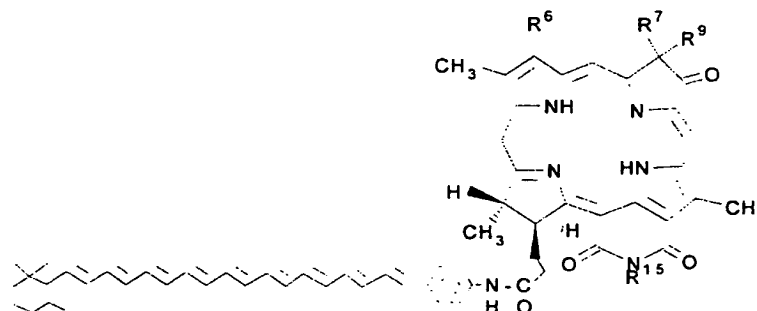
Where R^6 , R^7 , and R^9 are independently hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether or an amino acid residue; provided that, R^6 , R^7 , and R^9 together contain no more than a total of 16 carbon atoms and R^{15} is hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether, an amino acid residue or $-C(O)NHR^{18}$ where R^{18} is lower alkyl of from 1 to about 12 carbon atoms.

7.



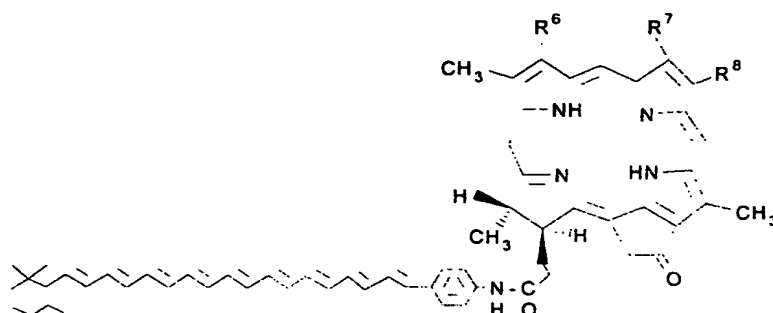
Where R^6 , R^7 , and R^9 are independently hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether or an amino acid residue; provided that, R^6 , R^7 , and R^9 together contain no more than a total of 16 carbon atoms and R^{15} is hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether, an amino acid residue or $-C(O)NHR^{18}$ where R^{18} is lower alkyl of from 1 to about 12 carbon atoms.

8.



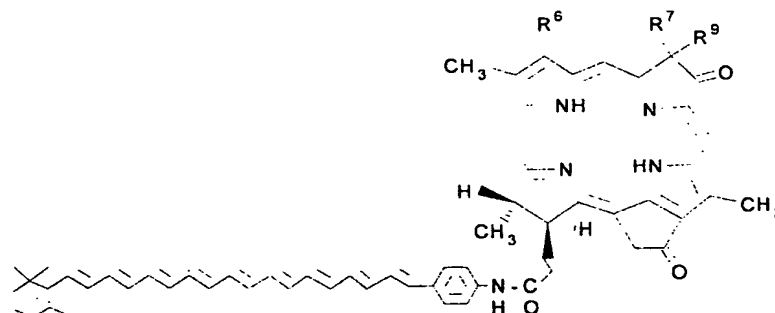
Where R^6 , R^7 , and R^9 are independently hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether or an amino acid residue; provided that, R^6 , R^7 , and R^9 together contain no more than a total of 16 carbon atoms and R^{15} is hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether, an amino acid residue or $-C(O)NHR^{18}$ where R^{18} is lower alkyl of from 1 to about 12 carbon atoms.

9.



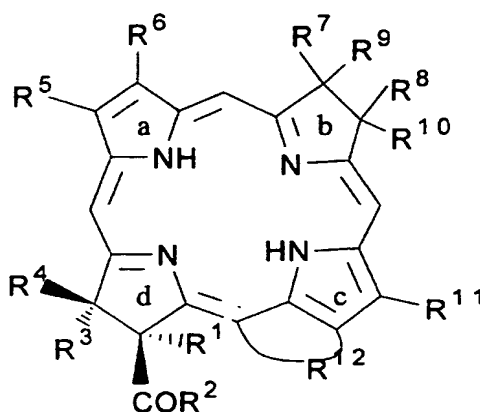
Where R^6 , R^7 , and R^8 are independently hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether or an amino acid residue; provided that, R^6 , R^7 , and R^8 together contain no more than a total of 16 carbon atoms and R^{15} is hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether, an amino acid residue or $-C(O)NHR^{18}$ where R^{18} is lower alkyl of from 1 to about 12 carbon atoms.

10.



Where R^6 , R^7 , and R^9 are independently hydrogen, alkyl, alkoxy, formyl, ketyl, alkenyl, alkylene alkyl ether or an amino acid residue; provided that, R^6 , R^7 , and R^9 together contain no more than a total of 16 carbon atoms.

Many photosensitizers, suitable as starting materials in accordance with the present invention for combination with carotene, have the formula:



where R^1 is hydrogen or methyl; R^2 is $-OH$; R^3 , R^4 and R^5 are independently, hydrogen, methyl or ethyl; R^6 and R^7 are independently $-R^{13}$, $-OR^{13}$, $-C(R^{16})(O)$, $-C(R^{16})_2OR^{13}$, $-CH=CHR^{13}$, or $-(CH_2)R^{14}$; R^8 is $-R^{13}$, $-OR^{13}$, $-C(R^{16})(O)$, $-C(R^{16})_2OR^{13}$, $-CH=CHR^{13}$, or $-(CH_2)R^{14}$ or taken with R^{10} is $=O$; R^9 is $-R^{13}$, $-OR^{13}$, $-C(R^{16})(O)$, $-C(R^{16})_2OR^{13}$, $-CH=CHR^{13}$, or $-(CH_2)R^{14}$ or taken with R^{10} is a chemical bond; R^{10} is $-R^{13}$, $-OR^{13}$, $-C(R^{16})(O)$, $-C(R^{16})_2OR^{13}$, $-CH=CHR^{13}$, or $-(CH_2)R^{14}$ or taken together with R^9 is a chemical bond or taken with R^8 is $=O$; R^{11} is R^{13} , or $-OR^{13}$; R^{12} is $-C(R^{13})_2C(Y)-$, $-C(O)O(O)C-$, $-C(NR^{13})O(O)C-$, or $-C(O)N(R^{15})-C(O)-$; R^{13} is, independently at each occurrence, hydrogen or lower alkyl of from 1 through about 10 carbon atoms; R^{14} is an amino acid residue, R^{15} is $-R^{13}$, $-R^{14}$, or $-C(O)NHR^{13}$; R^{16} is, independently at each occurrence, hydrogen or lower alkyl of 1 to about 4 carbon atoms and Y is $=O$, $=S$, or $2H-$.

HPPH, formula 9 above where R^6 is $-C(CH_3)O$ -hexyl, R^7 is $-CH_3$, R^8 is $-CH_2CH_3$ and the position of the carotene structure is substituted with $-COOH$, described in U.S. Patent 5,198,460 is reacted with amino carotene to form a carotene conjugate in accordance with the invention, as described below: Formula references are to those shown in the drawings.

The carboxylic acid functionality was first activated by preparing carbodiimide analog (*in situ*) before condensing with amino carotene. The structure of the conjugate was confirmed by NMR and mass spectrometry. The singlet oxygen production by HPPH and its carotene conjugate was measured in oxygen saturated toluene solutions by monitoring the singlet oxygen phosphorescence at 1270 nm. Ample production of singlet oxygen was detected in case of HPPH [singlet oxygen yield (Φ_{Δ}) = 0.45 and fluorescence yield (Φ_f) = 0.48].⁵ Under similar experimental conditions, the carotene conjugate did not produce singlet oxygen. Thus, a complete photoprotection was achieved.

Determination of tumor uptake by *in vivo* reflection spectroscopy:

The tumor uptake of the parent photosensitizer 2 (HPPH), Figure 1, and purpurin with a fused imide ring 4, Figure 2, were compared to the corresponding carotene conjugate 3 and 6 by *in vivo* reflection spectroscopy.

For these experiments, C3H mice with an axillary radiation induced fibrosarcoma (RIF) tumor were injected with the drug in an amount of 5 micro mole/Kg of body weight and the *in vivo* absorption spectra were taken at various time intervals. Figures 3 and 4 respectively show the uptake of HPPH 2 and purpurin-imide 4 at 24h post injection. Figures 5 and 6 show the uptake of the HPPH-carotene conjugate at 3h and 24h post injection. Compared to parent compound (HPPH, Fig. 3), the corresponding carotene conjugate shows a significant uptake of the drug in the tumor vs the skin fold. Similar enhancement in tumor uptake was observed with carotene conjugate of purpurin imide 6 as compared with the parent analog 4.

Preliminary *in vivo* Activity:

RIF tumors were implanted subcutaneously into the axilla of 5-7 week old female C3H mice. When tumors grew to about 5 mm diameter, mice were injected with photosensitizers at various doses. The mice (6 mice/group) were restrained in aluminum holders and each tumor illuminated with 135 J/cm² light from a laser tuned at the longest wavelength absorption maximum of the photosensitizers. The percentage of short-term control was recorded daily.

(A) Alkyl Ether Analogs of Purpurin-18 imides:

The preliminary *in vivo* activity of some of the compounds are summarized in Table 1. These data indicated that: (1) the hydrophobicity of the molecule can be varied by changing the length of the carbon chain (N-alkyl or -alkyl substituents), (ii) in some cases, compounds with same hydrophobic characteristics (same log p values), did not show similar activity; (iii) thus besides hydrophobicity, the steric and electronic factors also play important role in designing effective photosensitizers.

Among the compounds tested, the heptyl ether derivative of purpurin imide bearing N-hexyl substituent was found to be most effective at a dose of 1.0 μ mol/kg. The treatment and dose conditions for all the photosensitizers are briefly discussed in Table 1.

(B) Comparative *in vivo* activity of photosensitizers with and without carotene:

The *in vivo* photosensitizing activity of HPPH 2, and purpurin-imide 4 was compared with their corresponding carotene analogs, and the results are summarized in Table 2. As expected, compared to the non-carotenoid photosensitizers, carotene-conjugated photosensitizers were found to be ineffective. This is possibly due to the

carotene scavenging of singlet oxygen. Singlet oxygen is usually a necessary requirement for an effective photosensitizer.

Fluorescence Spectroscopy:

The fluorescence spectra of HPPH 2, purpurin-imide 4 and their corresponding carotene conjugates 3 and 6 were measured in dichloromethane. In the case of HPPH and its carotenoid analog, excitation at 660 nm gave strong emission at 670 nm. Similarly, purpurin imide and its carotenoid analog on excitation at 705 nm produced emission at 715 nm. Due to their strong fluorescence, and higher uptake in tumors, these compounds show great potential for use as diagnostic agents for malignant and non-malignant tumors.

Synthesis and Characterization of Carotene Conjugates:

HPPH-Carotene conjugate:

Hexyl ether analog of pyropheophorbide-a 2 was prepared by following the method reported previously (Pandey and Dougherty, U.S. Patent 5,198,460). Amino carotene (obtained from Mallinckrodt Medical Inc., St. Louis) was added into a solution of HPPH in dichloromethane containing a catalytic amount of dimethylaminopyridine (DMAP). The reaction mixture was stirred overnight. The solvent was evaporated, and the desired product was obtained by chromatography (Alumina/eluting with dichloromethane). Yield 65%. The structure of the conjugate was confirmed by NMR and mass spectrometry. See Figure 1.

Purpurin imide-carotene conjugate:

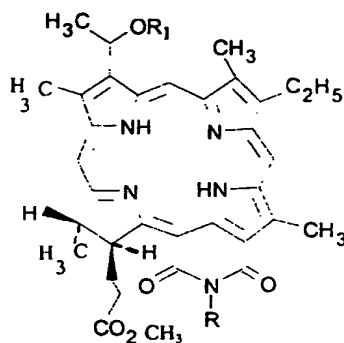
Methylpheophorbide-a was isolated from the alga *Spirulina Pacifica*, and reacted with alkyl amines. The intermediate amides as carboxylic acid analogs were

converted to the corresponding methyl esters, which on stirring with methanolic KOH at room temperature (5-10 min) produced the N-alkyl imide derivatives in 65-70% yield. The vinyl groups were then converted to various alkyl ether analogs by first reacting with 30% HBr/AcOH, and then with the desired alcohol (for example 4, Figure 2). The desired non-carotene conjugate analogs (Table 1) were prepared in high yield (70-75%). The methyl ester group (e.g., heptyl ether analog of N-hexyl purpurin imide 4) was hydrolyzed to the corresponding carboxylic acid, which was then converted into the respective carotene derivative by following the method discussed for the related HPPH-carotene conjugate. The structure of the conjugate was confirmed by NMR and mass spectrometry. The reaction sequences for the preparation of carotenoid analog 6 is shown in Figure 2.

HPPH and purpurine imide were used as being typical of the many carboxy containing porphyrin, chlorin and bacteriochlorin photosensitizers described in the background art. All of such photosensitizers are expected to form carotene conjugates in a manner similar to HPPH and purpurin imide.

Carotene conjugates of the invention are clearly useful in fluorescence spectroscopy for diagnosis of cancer without the photosensitizing characteristics of the base compounds.

Table 1. Preliminary *in vivo* Activity of Alkyl Ether Analogs of Purpurin-18 Imides



R ₁	R	Partition Coefficient (log P)	1-2	Tumor Response % [days]* 7	14	21	30	90
Methyl	Propyl	6.22		NR				
Propyl	Propyl	7.22	100	33	17	ongoing		
Methyl	Hexyl	7.72		NR				
Propyl	Hexyl (0.5 μmol)*	8.72		NR				
Hexyl	Propyl (0.5 μmol)*	8.72	100	66	33	17	17	17
Hexyl	Propyl	8.72	100	83	83	50	33	33
Heptyl	Hexyl (0.25 μmol)*	10.72	100	60	20	20	20	20
Heptyl	Hexyl	10.72	100	100	100	100	100	83

***Six mice/group (RIF tumor). 1.0 $\mu\text{mol/kg}$. 705 nm, 135J/cm² (24h post injection)**

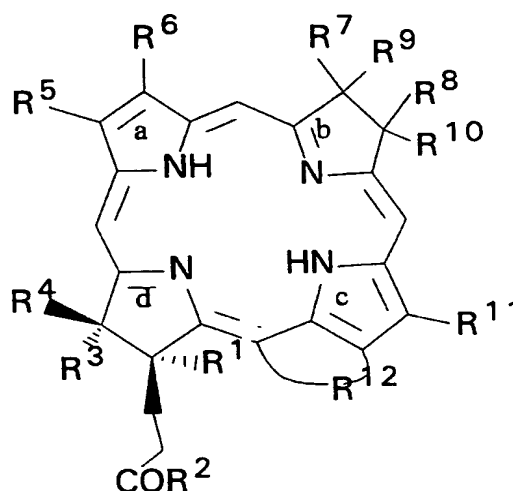
Table 2. Preliminary *In vivo* Activity of Photosensitizers
With and Without Carotene

Compound	Dose ($\mu\text{mol/kg}$)	Time Between inj. & treatment	Wavelength (nm) (for treatment)	%TUMOR RESPONSE (days)*				
				1-2	7	21	30	90
HPPH (2)	0.47	24	665	100	100	80	50	50
HPPH- carotene (3)	0.47	24	665	NO RESPONSE				
Purpurin- imide (4)	1.0	24	705	100	100	100	100	83 Φ
Purpurin- imide- carotene (6)	1.0	24	705	NO RESPONSE				

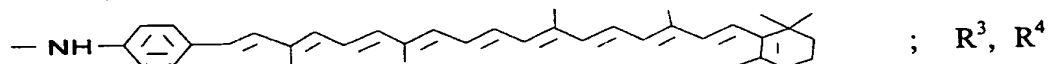
*Six mice/group (RIF tumor). 135J/cm^2 Φ 5/6 mice were tumor free on day 90

What is claimed is:

1. A carotene conjugate of a photosensitizer selected from the group consisting of porphyrins, chlorins and bacteriochlorins.
2. A photosensitizer of the formula:



where R^1 is hydrogen or methyl; R^2 is:



and R^5 are independently, hydrogen, methyl or ethyl; R^6 and R^7 are independently - R^{13} , $-OR^{13}$, $-C(R^{16})(O)$, $-C(R^{16})_2OR^{13}$, $-CH=CHR^{13}$, or $-(CH_2)R^{14}$; R^8 is $-R^{13}$, $-OR^{13}$, $-C(R^{16})(O)$, $-C(R^{16})_2OR^{13}$, $-CH=CHR^{13}$, or $-(CH_2)R^{14}$ or taken with R^{10} is $=O$; R^9 is $-R^{13}$, $-OR^{13}$, $-C(R^{16})(O)$, $-C(R^{16})_2OR^{13}$, $-CH=CHR^{13}$, or $-(CH_2)R^{14}$ or taken with R^{10} is a chemical bond; R^{10} is $-R^{13}$, $-OR^{13}$, $-C(R^{16})(O)$, $-C(R^{16})_2OR^{13}$, $-CH=CHR^{13}$, or $-(CH_2)R^{14}$ or taken together with R^9 is a chemical bond or taken with R^8 is $=O$; R^{11} is R^{13} , or $-OR^{13}$; R^{12} is $-C(R^{13})_2C(Y)-$, $-C(O)O(O)C-$, $-C(NR^{13})O(O)C-$, or $-$

$C(O)N(R^{15})-C(O)-$; R^{13} is, independently at each occurrence, hydrogen or lower alkyl of from 1 through about 10 carbon atoms; R^{14} is an amino acid residue, R^{15} is $-R^{13}$, $-R^{14}$, or $-C(O)NHR^{13}$; R^{16} is, independently at each occurrence, hydrogen or lower alkyl of 1 to about 4 carbon atoms and Y is $=O$, $=S$, or $2H-$.

3. The compound of claim 2 wherein R^1 is H, R^3 is $-CH_3$, R^4 is H, R^5 is CH_3 , R^6 is $-CH(CH_3)O(CH_2)_6CH_3$, R^7 is $-CH_3$, R^8 is $-CH_2CH_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-CH_3$ and R^{12} is $-C(O)CH_2-$.

4. The compound of claim 2 wherein R^1 is H, R^3 is $-CH_3$, R^4 is H, R^5 is CH_3 , R^6 is $-CH(CH_3)O(CH_2)_6CH_3$, R^7 is $-CH_3$, R^8 is $-CH_2CH_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-CH_3$ and R^{12} is $-C(O)N((CH_2)_5CH_3)CH_3-$.

5. The compound of claim 2 wherein R^1 is H, R^3 is $-CH_3$, R^4 is H, R^5 is CH_3 , R^6 is $-CH(CH_3)O(CH_2)_6CH_3$, R^7 is $-CH_3$, R^8 is $-CH_2CH_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-CH_3$ and R^{12} is $-C(O)N((CH_2)_3CH_3)CH_3-$.

6. The compound of claim 2 wherein R^1 is H, R^3 is $-CH_3$, R^4 is H, R^5 is CH_3 , R^6 is $-CH(CH_3)OCH_3$, R^7 is $-CH_3$, R^8 is $-CH_2CH_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-CH_3$ and R^{12} is $-C(O)N((CH_2)_2CH_3)C(O)-$.

7. The compound of claim 2 wherein R^1 is H, R^3 is $-CH_3$, R^4 is H, R^5 is CH_3 , R^6 is $-CH(CH_3)O(CH_2)_2CH_3$, R^7 is $-CH_3$, R^8 is $-CH_2CH_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-CH_3$ and R^{12} is $-C(O)N((CH_2)_2CH_3)C(O)-$.

8. The compound of claim 2 wherein R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is CH_3 , R^6 is $-\text{CH}(\text{CH}_3)\text{OCH}_3$, R^7 is $-\text{CH}_3$, R^8 is $-\text{CH}_2\text{CH}_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}((\text{CH}_2)_5\text{CH}_3)\text{C}(\text{O})-$.
9. The compound of claim 2 wherein R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is CH_3 , R^6 is $-\text{CH}(\text{CH}_3)\text{O}(\text{CH}_2)_2\text{CH}_3$, R^7 is $-\text{CH}_3$, R^8 is $-\text{CH}_2\text{CH}_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}((\text{CH}_2)_5\text{CH}_3)\text{C}(\text{O})-$.
10. The compound of claim 2 wherein R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is CH_3 , R^6 is $-\text{CH}(\text{CH}_3)\text{O}(\text{CH}_2)_5\text{CH}_3$, R^7 is $-\text{CH}_3$, R^8 is $-\text{CH}_2\text{CH}_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}((\text{CH}_2)_2\text{CH}_3)\text{C}(\text{O})-$.
11. The compound of claim 2 wherein R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is CH_3 , R^6 is $-\text{CH}(\text{CH}_3)\text{O}(\text{CH}_2)_5\text{CH}_3$, R^7 is $-\text{CH}_3$, R^8 is $-\text{CH}_2\text{CH}_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}((\text{CH}_2)_5\text{CH}_3)\text{C}(\text{O})-$.
12. The compound of claim 2 wherein R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is CH_3 , R^6 is $-\text{CH}_2\text{CH}_2\text{OR}^{17}$, R^7 is $-\text{CH}_3$, R^8 is $-\text{CH}_2\text{CH}_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}((\text{CH}_2)_5\text{CH}_3)\text{C}(\text{O})-$ and R^{17} is primary or secondary alkyl containing 1 to about 20 carbon atoms.
13. The compound of claim 2 wherein R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is $-\text{CH}_3$, R^6 is $-\text{CH}(\text{CH}_3)\text{OR}^{17}$, R^7 is $-\text{CH}_3$, R^8 is $-\text{CH}_2\text{CH}_3$, R^9 and R^{10} together form a chemical bond, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}((\text{CH}_2)_5\text{CH}_3)\text{C}(\text{O})-$ and R^{17} is primary or secondary alkyl containing 1 to about 20 carbon atoms.

14. The compound of claim 2 wherein R^1 is H, R^3 is $-\text{CH}_3$, R^4 is H, R^5 is CH_3 , R^6 is lower alkyl of 1 to 4 carbon atoms or a formal or carbonyl containing group of 1 to 4 carbon atoms, R^7 is $-\text{CH}_3$, R^8 is H, $-\text{OR}^{13}$ or with R^{10} is $=\text{O}$, R^9 is H, $-\text{OR}^{13}$ or with R^{10} forms a chemical bond, R^{10} is ethyl or with R^9 forms a chemical bond or with R^8 is $=\text{O}$, R^{11} is $-\text{CH}_3$ and R^{12} is $-\text{C}(\text{O})\text{N}(\text{R}^{13})\text{C}(\text{O})-$ or $-\text{C}(\text{O})\text{O}(\text{O})\text{C}-$.
15. The compound of claim 2 wherein R^1 is hydrogen, R^3 is methyl, R^4 is hydrogen, R^5 is methyl, R^6 is $-\text{CH}(\text{CH}_3)\text{OR}^{13}$, R^7 is methyl, R^8 is ethyl, R^9 and R^{10} together form a chemical bond; R^{11} is methyl; and R^{12} is $-\text{CH}_2\text{C}(\text{O})-$.
16. The compound of claim 2 wherein R^1 is hydrogen, R^3 is methyl, R^4 is hydrogen, R^5 is methyl, R^6 is $-\text{CH}=\text{CH}_2$, R^7 is methyl, R^8 is ethyl, R^9 and R^{10} together form a chemical bond; R^{11} is methyl; and R^{12} is $-\text{CH}_2\text{C}(\text{O})-$.
17. The compound of claim 2 wherein R^1 is hydrogen, R^3 is methyl, R^4 is hydrogen, R^5 is methyl, R^6 is $-\text{CH}(\text{CH}_3)\text{O}(\text{CH}_2)_5\text{CH}_3$, R^7 is methyl, R^8 is ethyl, R^9 and R^{10} together form a chemical bond; R^{11} is methyl; and R^{12} is $-\text{CH}_2\text{C}(\text{O})-$.
18. The compound of claim 2 wherein R^1 is hydrogen, R^3 is methyl, R^4 is hydrogen, R^5 is methyl, R^6 is $-\text{CH}=\text{CH}_2$, R^7 is methyl, R^8 is ethyl, R^9 and R^{10} together form a chemical bond; R^{11} is methyl; and R^{12} is $-\text{C}(\text{O})\text{OC}(\text{NR}^{13})-$.
19. The compound of claim 2 wherein R^1 is hydrogen, R^3 is methyl, R^4 is hydrogen, R^5 is methyl, R^6 is $-\text{CH}=\text{CH}_2$, R^7 is methyl, R^8 is ethyl, R^9 and R^{10} are hydrogen; R^{11} is methyl; and R^{12} is $-\text{C}(\text{O})\text{OC}(\text{NR}^{13})-$.

20. A method for diagnosis of cancer which comprises injecting a compound of claim 1 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.
21. A method for diagnosis of cancer which comprises injecting a compound of claim 2 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.
22. A method for diagnosis of cancer which comprises injecting a compound of claim 3 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.
23. A method for diagnosis of cancer which comprises injecting a compound of claim 4 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.
24. A method for diagnosis of cancer which comprises injecting a compound of claim 5 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.

25. A method for diagnosis of cancer which comprises injecting a compound of claim 6 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.

26. A method for diagnosis of cancer which comprises injecting a compound of claim 7 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.

27. A method for diagnosis of cancer which comprises injecting a compound of claim 8 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.

28. A method for diagnosis of cancer which comprises injecting a compound of claim 9 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.

29. A method for diagnosis of cancer which comprises injecting a compound of claim 10 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.

30. A method for diagnosis of cancer which comprises injecting a compound of claim 11 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.
31. A method for diagnosis of cancer which comprises injecting a compound of claim 12 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.
32. A method for diagnosis of cancer which comprises injecting a compound of claim 13 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.
33. A method for diagnosis of cancer which comprises injecting a compound of claim 14 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.
34. A method for diagnosis of cancer which comprises injecting a compound of claim 15 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.

35. A method for diagnosis of cancer which comprises injecting a compound of claim 16 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.

36. A method for diagnosis of cancer which comprises injecting a compound of claim 17 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.

37. A method for diagnosis of cancer which comprises injecting a compound of claim 18 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.

38. A method for diagnosis of cancer which comprises injecting a compound of claim 19 into an animal at a dosage of between 1 and 10 micromoles per kilogram of body weight of the animal and detecting locations of concentrated uptake of said compound by means of spectroscopy.

1/6

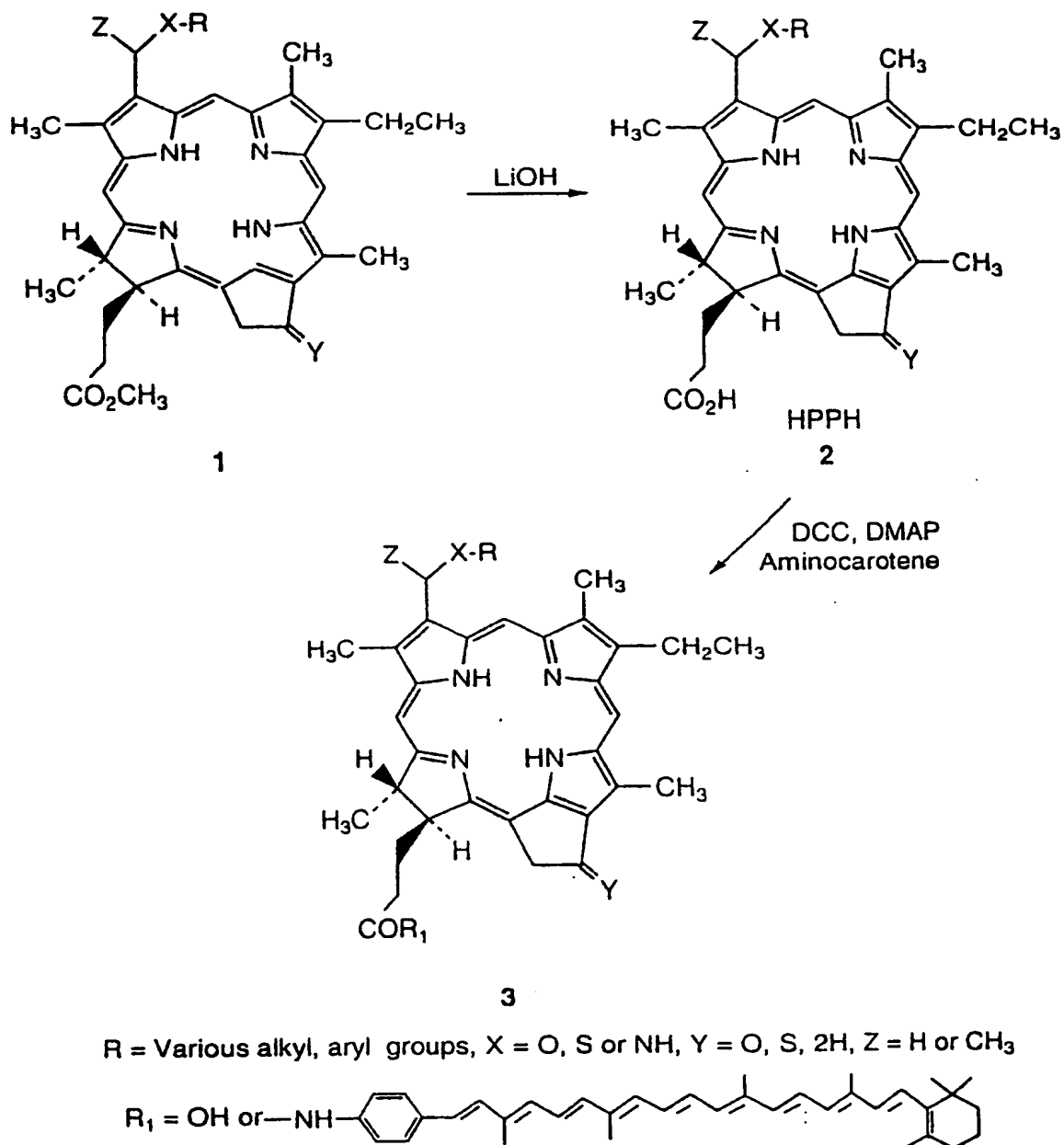


FIGURE 1

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2/6

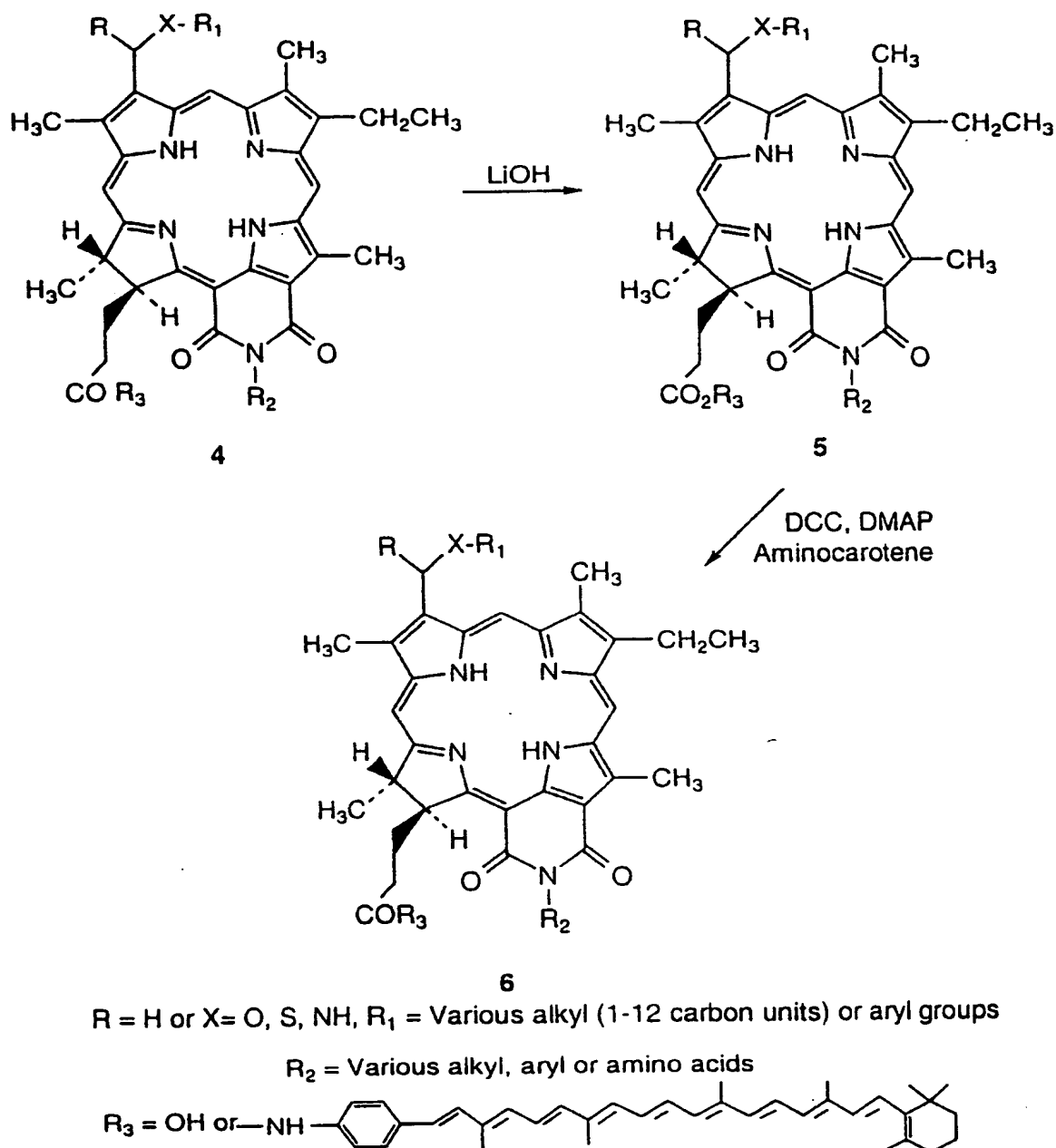
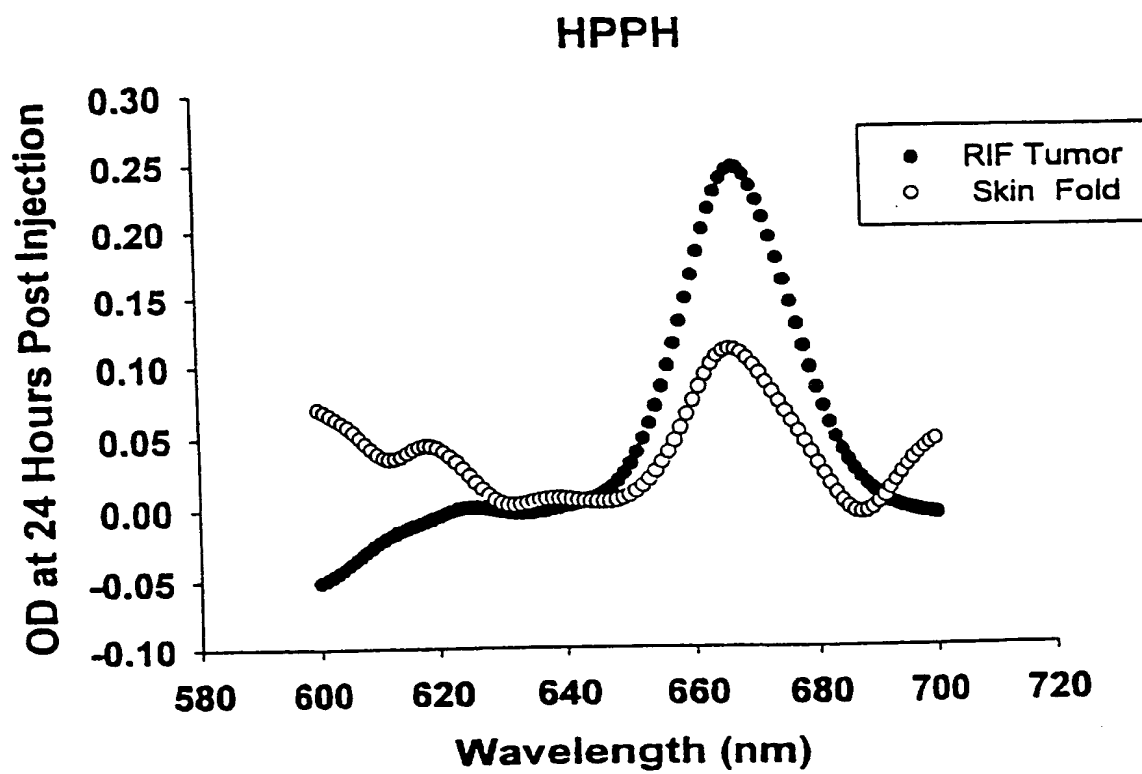


FIGURE 2

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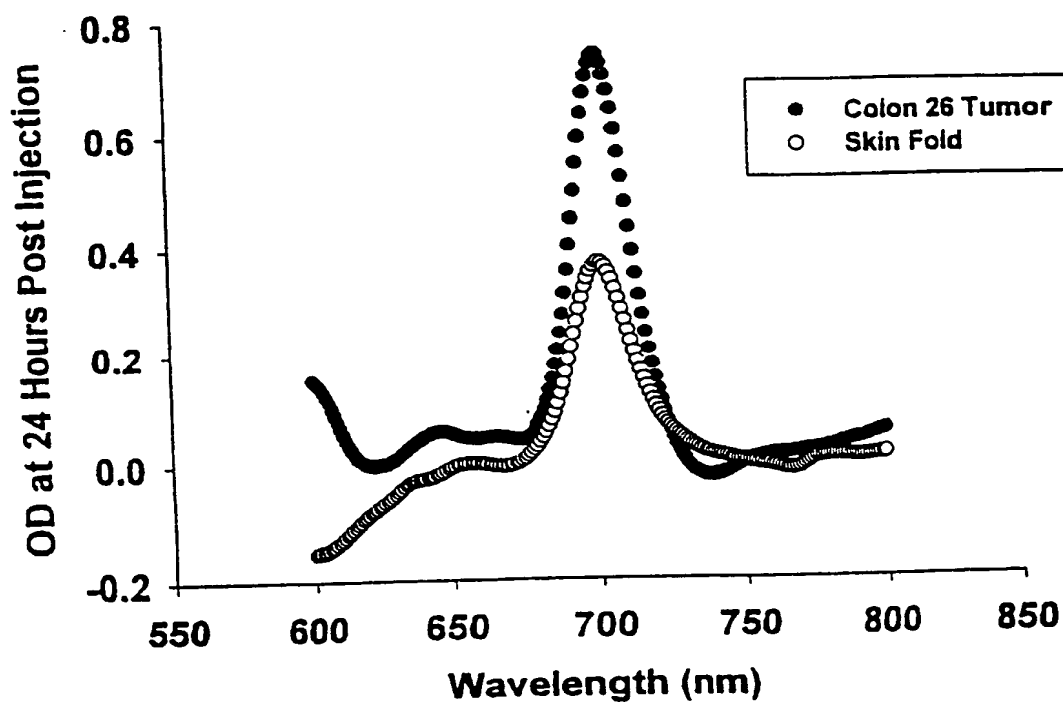
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hph 24 hours2.jbn

**FIGURE 3***SUBSTITUTE SHEET (RULE 26)*

4/6

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Purpurin-Imide**FIGURE 4**

5/6

Carotene Conjugate of HPPH

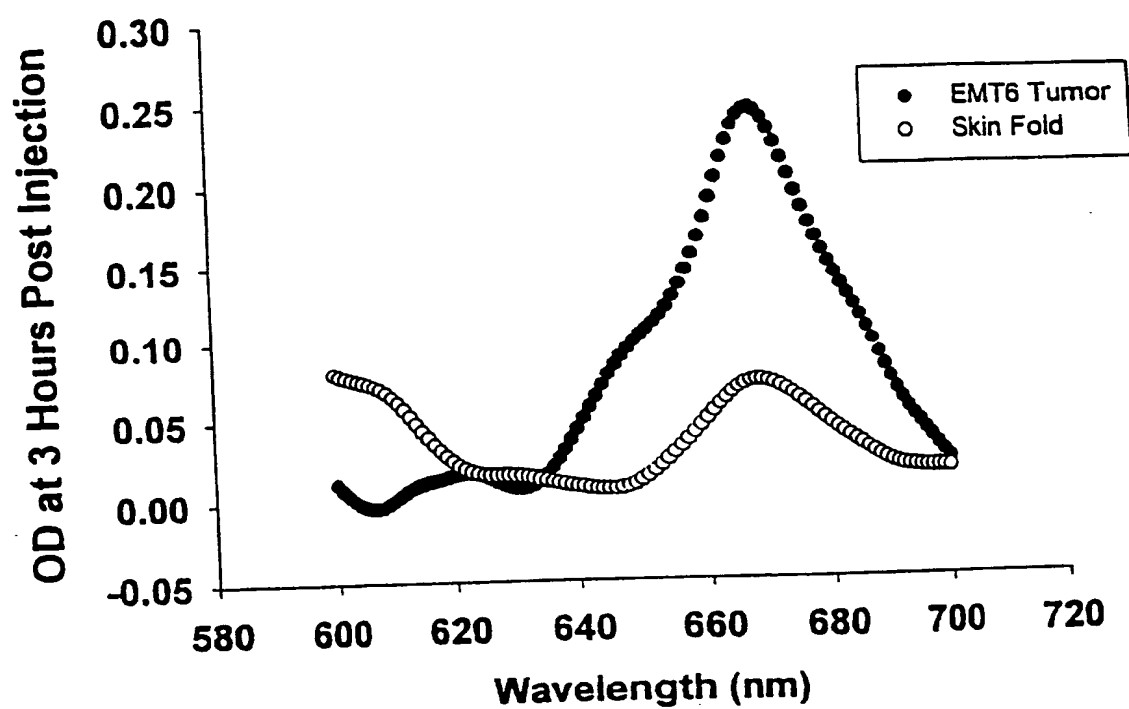
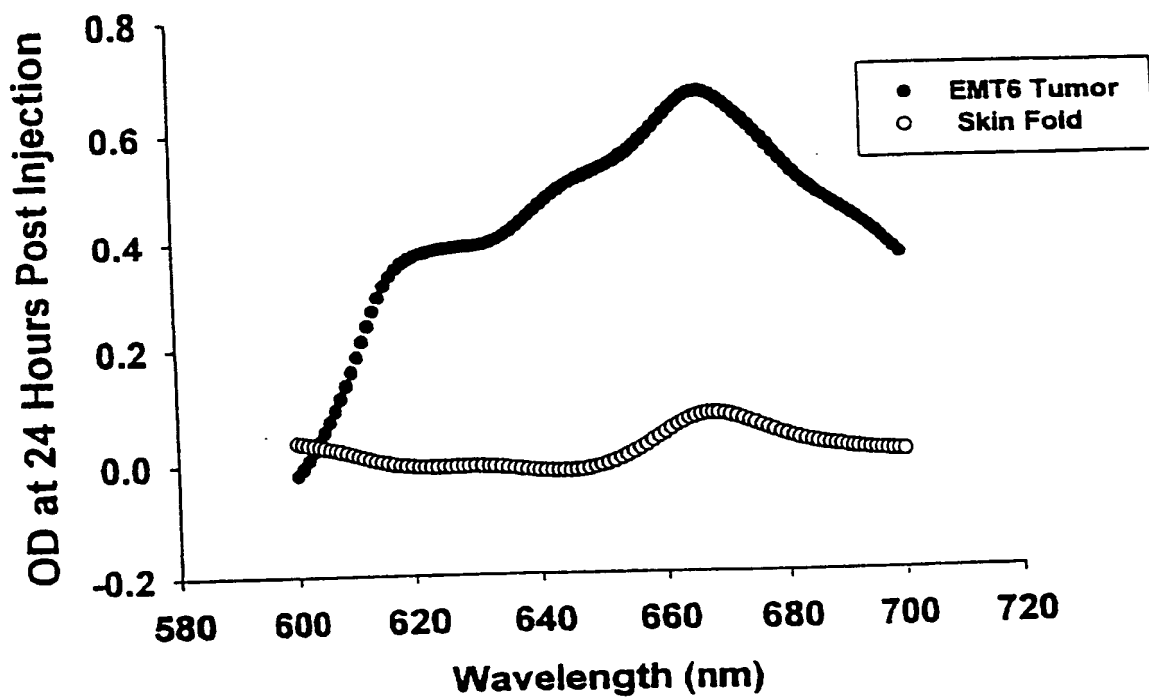


FIGURE 5

6/6

Carotene Conjugate of HPPH**FIGURE 6**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12170

A. CLASSIFICATION OF SUBJECT MATTER IPC(6) :C07D 487/22; A61K 31/40 US CL :540/145; 514/410 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 540/145; 514/410 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) APS, CAS ONLINE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,238,940 A (LIU et al.) 24 August 1993, see entire document.	1N
X	US 5,591,847 A (PANDEY et al.) 07 January 1997, see column 4, lines 33-67.	1, 2, 14, 33
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Y		1, 2, 14, 33
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art *G* document member of the same patent family		
Date of the actual completion of the international search 02 SEPTEMBER 1999		Date of mailing of the international search report 21 OCT 1999
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